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Industrial *Cannabis sativa* (Hemp) seeds used as Probiotic Energy Milk Drink-An Update

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Abstract

Probiotics are living microorganisms that have many beneficial functions to the human body, such as regulating glucose and lipid metabolism, enhancing immunity, relieving constipation, and reducing inflammation. Hemp milk is a quality product from the point of view of nutraceutical value. Among the prime reasons why hemp milk has been gaining momentum is due to its positive nutritional balance. Unlike other seed milks such as almond or soy milk, hemp milk contains all nine of the essential amino acids and is, therefore a complete vegetarian and vegan protein source for people with dietary needs. Hemp seeds are rich in protein content (25-30%) and the source of **Omega-3 and Omega-6**. Hemp is rich in protein and vitamins such as A and E, it is a viable alternative to cow milk not only for those who want to change the diet in a healthy way or have made a vegan choice but also for lactose intolerants. Hemp milk, with its highly nutritional value and low allergencity, looks to be an attractive alternative to dairy, soy, and nut milks. Comparison of milk products in the market reveals hemp milk as a better source of minerals than other dairy and plant milks. Hemp milk has a unique texture and flavor profile that is very distinct from the rest of the alternatives, such as almond milk or oat milk. Increasing demand for plant-based milk has enlarged its industry due to lactose intolerance, cow's milk allergy, and vegan lifestyles.

Keywords: Hemp milk; Probiotic; *Lactobacillus plantarum*; *Lactobacillus fermentum*; *Bifidobacterium bifidum*; Functional food; Milk substitute

1. Introduction

Cannabis sativa has been used for thousands of years for recreational, medicinal, or religious purposes. Now a days *Cannabis sativa* has been domesticated, cultivated and distributed throughout the world. *Cannabis sativa* is a flowering plant from the *Cannabaceae* family and genus cannabis[1-52]. *Cannabis sativa* has a long history in India, recorded in legends and religion. It was found in various habitats ranging from sea level to the temperate and alpine foothills of the Indian Himalaya Region from where it was probably spread over the last 10,000 years [1-52]. Many historians believed that Indian Himalayan Region was the centre of origin of *Cannabis sativa* L. and *Cannabis indica* L. [1-52]. *Cannabis sativa* is a plant known for narcotic substance of notorious psychoactive effect, but when used correctly, it provides a plethora of medicinal benefits [1-52]. Industrial *Cannabis sativa* (Hemp or fibre type) can also be consumed as a cannabis tea in remote Himalayan villages of India [1-52]. In remote area, the use of *Cannabis sativa* is totally depends on traditional knowledge, which transmitted through family traditions basically through oral conversations [1-52]. *Cannabis sativa* is also a wild noxious weed with notorious psychoactive principle, Λ9-tetrahydrocannabinol (THC) found growing in India, China, Bhutan, Nepal, Pakistan, Afghanistan, Iran, and Morocco [1-52]. Tribal people in the Indian Himalayan region used *Cannabis sativa* as a home made herbal medicine for many diseases [1-52]. During, Covid-19, the infusion of *Cannabis sativa* flower with a morning cup of tea has saved the life of many people [1-52]. Industrial *Cannabis sativa*

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(Hemp or fibre type) oil was used as dengue mosquito repellent for controlling dengue viral fever, bacterial infections and fungal diseases [1-52].

Cannabis sativa L., is classified into two types as Industrial *Cannabis sativa*, (hemp or fiber type) and Medical *Cannabis sativa* L.(drug or marijuana) based on its THC content[1-52]. Medical *Cannabis sativa* (drug or marijuana) contains a very high levels of THC (above 0.3 to 38% of dry weight) [1-52, 80]. On the other hand Industrial *Cannabis sativa*, (hemp or fiber type) contains very low levels of THC (0 to 0.3% of dry weight) [1-52]. However, due to the presence of psychoactive molecules, Λ9-tetrahydrocannabinol (Λ9-THC) and Λ8-tetrahydrocannabinol (Λ8-THC), *Cannabis sativa* cultivation and its use is restricted/regulated in many countries [1-52, 80]. Industrial *Cannabis sativa*, (hemp or fiber type), as a diverse plant, can be a revolutionary crop for a better future and for upcoming generations[1-25]. It is an eco-friendly and worthwhile crop that complements a sustainable growth system. Industrial *Cannabis sativa*, (hemp or fiber type) farming has the potential to dramatically minimize the amount of carbon impact on the environment and can be cultivated with a little or no usage of chemical pesticides or fertilizers [1-52]. The stalks, seeds, and leaves are converted into various construction materials, textiles, paper, food, furniture, cosmetics, and healthcare products [1-52]. Biochar, bioplastics, biofuels, and biopesticides are some of the innovative applications of the Industrial *Cannabis sativa*, (hemp or fiber type) which are subjects of research and debate at present time [1-52]. In addition to fiber and essential oils, the hemp plant produces hundreds of secondary metabolites including flavonoids, diterpenes, triterpenes, and cannabinoids [1-52]. Unlike primary metabolites such as proteins, nucleic acids, lipids, and carbohydrates that are essential for life, secondary metabolites benefit plants in other ways such as deterring predators, attracting pollinators, or preventing infection [1-52]. Several secondary metabolites that are unique to *Cannabis sativa* L. include some Cannflavins (flavonoids), Cannabisins (lignans), and Cannabinoids [1-52]. Hemp produces over 100 Cannabinoids including Cannabidiol (CBD), Cannabidiolic acid (CBDA), the psychotropic Cannabinoid, Λ9- tetrahydrocannabinol (Λ9-THC), Λ9-tetrahydrocannabinolic acid-A (Λ9-THCA-A), Cannabigerol (CBG), Cannabigerolic acid (CBGA), and Λ9-tetrahydrocannabutol [1-52]. Despite legal restrictions, Cannabinoids and other compounds from hemp have a long and extensive history of safe use in humans. These products have been administered orally, sublingually, dermally, and by inhalation[1-52]. As a class of natural products, cannabinoids have been shown to have suitable oral bioavailability, metabolism, blood–brain barrier permeability, and safety for use as therapeutic agents [1-52]. Based on the established therapeutic efficacy of Cannabidiol (CBD) and Λ9-tetrahydrocannabutol (Λ9- THC), these and other cannabinoids are under investigation for additional pharmacological activities, including activity as antiviral agents [1-52]. Among the vast range of compounds, multiple research papers have shown that cannabinoids, such as Cannabidiol and Λ-9-tetrahydrocannabinol, have antiviral effects[1-52]. There is limited evidence that demonstrates the therapeutic effects of Cannabinoids in viral infections [1-52]. Some scientists believed that Λ9-tetrahydrocannabutol (Λ9- THC), may be advantageous in viral illnesses, when the inflammatory response of the host is pathogenic. The antiinflammatory properties of cannabinoids were found to be associated with the treatment of COVID-19 [1-52].

Hemp milk is made by blending hemp seeds with water and then straining the mixture to create a creamy, smooth milk alternative [53- 77]. It is among the best plant milks to balance with an assortment of benefits spanning from being a good source of omega-3 and omega-6 fatty acids, and necessary amino acids, to minerals including magnesium, iron, and calcium[53- 77]. Among the prime reasons why hemp milk has been gaining momentum is due to its positive nutritional balance[53- 77]. Unlike other seed milks such as almond or soy milk, hemp milk contains all nine of the essential amino acids and is, therefore a complete vegetarian and vegan protein source for people with dietary needs [53- 77].

2. Industrial *Cannabis sativa*, (hemp or fiber type): Seed Protein

Hemp seeds are rich in protein content (25-30%) and majorly used as birdseed and animal feed [1-57]. The bird and fish feed are important markets for hemp seeds in animal nutrition [1-57]. Fish and birds need fatty acids with a high share of **Omega-3 and Omega-6** fatty acids for optimum development [1-57]. Hemp seed is a balanced health product with bioactive components that have the capacity to aid health beyond that of basic nutrition [1-57]. The major constituents of hemp seed include easily digestible protein (20–25%), polyunsaturated fatty acid (PUFA), abundant lipids (25–35%), and carbohydrates (20–30%) high in insoluble fibre [1-57]. Hemp seed protein is well-suited for human and animal consumption, consisting mainly of high-quality, easily digestible proteins edestin, and albumin, which are abundant with essential amino acids [1-57]. The rich source of PUFA, linoleic acid (LA; omega-6) and alpha-linolenic acid (ALA; omega-3), is favorable and regarded as balanced for human nutrition at a ratio of 3:1 (1-21). Nutritional recommendations indicated that 15–20% of daily caloric intake should come from fats, and approximately one-third of these fats should be essential fatty acids in a 3:1 ratio [1-57]. It is estimated that this dietary goal can be met with three tablespoons of hemp seed oil [1-57]. Numerous health benefits and potential therapies are reported for hemp seed [1-57]. Hemp seed delivers a desirable ratio of **Omega-6 to Omega-3** PUFA, which can improve cardiovascular health, reduce osteoporosis symptoms, and diminish eczema conditions [1-57]. Cannabidol (CBD) exerts

pharmacological properties that make it a potential therapeutic agent for central nervous system diseases, such as epilepsy, neurodegenerative diseases, and multiple sclerosis [1-57]. The hemp seeds and sprouts to be rich in beneficial bioactive compounds with both in vitro and ex vivo antioxidant activities [1-57].

Furthermore, these compounds exhibited an anti-mutagenic effect on *Saccharomyces cerevisiae* [1-57]. The main polyphenols identified in seeds and sprouts exhibiting antioxidant activities were Cannabisin A, B, C, and caffeoyltyramine [1-57]. The two primary compounds identified in sprouts that provide nutraceutical benefits were linoleic acid and gluconic acids, which act as intermediaries in the production of vitamin C [1-57]. Terpenes, which are also found in hemp, have anti-inflammatory and some anti-allergic properties, can treat pain, prevent the production of ROS, and act as potent antioxidants [1-57]. Due to the presence of a wide variety of nutrients, including high levels of PUFA and essential amino acids, hemp seeds are praised for providing adequate quantities of different nutrients to satisfy human dietary requirements [1-57]. Its endogenous omega fatty acids are also being credited for their potential health benefits to cardiovascular health, inflammatory fighting, and cognitive functioning. Hemp milk is also lactose-free, gluten-free, and in most instances, contains added vitamins such as vitamin D and B12, making it a suitable option for individuals with food intolerance or just those who follow a dairy-free diet.

3. Probiotic Hemp Milk

Probiotics are living microorganisms that have many beneficial functions to the human body, such as regulating glucose and lipid metabolism, enhancing immunity, relieving constipation, and reducing inflammation [53-63-76, 83-85]. They have a wide application in fermented milk products, and it was found that they could improve the flavor and enhance the beneficial functions of fermented milk. However, numerous studies have shown that unique aromas and compounds depend on the probiotics used [53-63]. *L. rhamnosus*, *L. paracasei*, and *L. plantarum* are commonly used probiotics in fermented milk, which have been successfully applied in soy fermented milk [53-63-77]. The current definition of a probiotic means those microbial strains that positively affect consumer health when taken in the right amount. Accordingly to FAO/WHO guidelines, the count of probiotic bacteria can not be less than the value corresponding to 10^6 cfu per 1 mL of a product through the entire period of its storage till the end of its shelf life. This value has been deemed the therapeutic minimum [53-79, 83-85].

In recent years, there is an increasing trend in the consumption of hemp or food products with hemp/hemp proteins as functional ingredients, due to its recognized nutritive value, excellent digestibility, and low allergenicity [53-77]. Hemp milk is a quality product from the point of view of nutraceutical value. The type of nutrient composition appeals to health-conscious consumers wishing to supplement their diets with natural, plant-based alternatives that are good for general health [53-77]. Hemp milk, produced from whole hemp seeds, contains most of the original nutrients and has been reported to perform certain health benefits, such as the reduction of serum cholesterol, triacylglycerol, and thyroid hormones [53-76]. Besides, because it is lactose free and hypoallergenic nature, hemp milk might be an attractive alternative to dairy, soy, and nut milks [53-77]. The functional beverage category, which offers beverages that contribute to additional benefits of health besides general nutrition, is the most innovative development within the hemp milk industry [53-77]. Historically used as a milk alternative, hemp milk is becoming an integral part of functional categories within beverages, i.e., fortified beverages, immune-boosting products, and gut-friendly formulations [53- 77]. Manufacturers are blending hemp milk with other nutrients like probiotics, prebiotics, plant proteins, and adaptogens to satisfy the growing demand for beverages that contribute to overall wellness [53- 77]. The rise of functional hemp milk beverages is a result of increased consumer awareness of holistic health and preventive medicine [53- 77]. As the plant-based beverage market experiences a high growth rate, consumers are no longer satisfied with the plain, unflavored version [53- 77]. Flavored hemp milk satisfies this need by being available in a range of flavors, from vanilla and chocolate to seasonal varieties such as pumpkin spice or coconut, and is thus an easy ingredient to add to anything from smoothies to desserts. That convenience has rendered it a sought-after addition to the retail store shelves, with numerous products to cater to personal taste and dietary requirements [53- 77]. These innovations offer consumers holistic, quality product that is great tasting and serves a purpose toward one's health objective, be it enhancing immune response, promoting digestive balance, or bone wellness [53- 77].

Hemp milk is obtained by grinding the seeds. It is the same variety used to obtain the flour pastry, having sweet flavour, and oil [53-76]. Hemp is rich in protein and vitamins such as A and E, it is a viable alternative to cow milk not only for those who want to change the diet in a healthy way or have made a vegan choice but also for lactose intolerants [53-76]. Being free from sugar naturally contained in animal milk it is more digestible [53-76]. The hemp milk contains about 30% of the recommended daily vitamin D ratio and a good amount of calcium, two important substances for the robustness of the skeleton that are present in cow milk but naturally absent in vegetable ones (almond, coconut, oats) to which they are usually added [53-76]. The hemp drink is an excellent source of minerals such as magnesium, potassium, and iron. As hemp milk is derived from hemp seeds, the positive effects of this product on

human health depend on the amount of linolenic acid (C18:2 ω-6) and α-linolenic acid (C18:3 ω-3) [53-76]. The properties of high amount of PUFAs are beneficial for all the population, by reducing the accumulation of bad cholesterol, regulate the pressure activate neurons, and promoting mental efficiency[53-76].

Recently in one of the study by Zhou et al., (2024) [62] reported that fermented milk has been used as human food for a long time. It is a dairy product with a unique flavor made by fermenting fresh milk as a raw material [62-76]. It has an acidic flavor and is easy to solidify. It has a huge market worldwide because it is more nutritious and has a richer flavor than fresh milk[62-76]. Traditional fermented milk is usually made by inoculating lactic acid bacteria (LAB) for fermentation [53-62]. Consumers are attracted not only by its slightly sour and creamy taste but also by its associated health benefits. However, fermented milk may cause lactose intolerance, animal protein allergy, and cardiovascular disease caused by high saturated fat content in some individuals, which limits the consumption of fermented milk worldwide[62]. In addition, the amount of animal protein is insufficient to meet the daily needs of humans in developing countries. Based on these, plant-based fermented milk as a non-dairy substitute has become a preferred choice for consumers and a research hotspot in the food and beverage industry [62-76].

Hemp seeds are derived from ripe and dried hemp fruit and have been an edible food ingredient for thousands of years [1-63-76]. They are rich in nutrients, including 25 to 35% oil, 20 to 30% carbohydrates, 20 to 25% protein, 10 to 15% insoluble fiber, and vitamins and minerals such as potassium, magnesium, phosphorus, calcium, sulfur, iron, and zinc [1-63]. Of these, 85% of the fatty acids in hempseeds are polyunsaturated, particularly the ratio of linoleic acid (ω-6) and α-linolenic acid (ω-3) was between 2:1 and 3:1, which is the ideal ratio for optimal health and has been effectively developed and utilized [61]. 11S globulin and 2S albumin are the main proteins in hempseeds, containing all the essential amino acids (with good digestibility) in the content and proportions required for human dietary needs [61]. It is worth mentioning that arginine in hemp protein accounts for 12%, which is the highest content of all plant seed proteins [62]. Arginine, an endogenous amino acid, is involved in the synthesis of various products responsible for regulatory functions in the body [62], with a special application value in the development of functional food. High protein content and such nutritional properties made defatted hemp an ideal raw material for plant-based fermented milk preparation [62]. Hence, defatted hemp has received high attention as a raw material in the development of functional fermented milk in recent years [62]. The large amounts of acetate, propionate, and butyrate produced during the fermentation process of hemp seed beverages are beneficial for the growth of probiotics and have strong prebiotic activity [62]. In fermented hemp milk containing kefir grains, there was an increase in the content of nutrients such as organic acids, free fatty acids, and amino acids, as well as in the type and concentrations of flavor compounds such as alcohols, acids, and esters. Compared to unfermented products, the texture of the final product, including particle size, viscosity, and resistance to phase separation, was also increased [62]. The above results confirmed the potential application of hemp seed in the preparation of functional fermented milk [62].

The creation of hemp milk creamers is a trend in the global hemp milk industry, specially in the coffee and beverage sectors[53-63-77]. It is rich and creamy and an excellent replacement for plant-based creamers and coffee substitutes. With a growing number of consumers turning towards dairy-free consumption, there has been a bigger demand for alternatives to coffee and tea that would provide the same amount of creaminess as dairy[53-63-77]. Hemp milk has a unique texture and flavor profile that is very distinct from the rest of the alternatives, such as almond milk or oat milk. Increasing demand for plant-based milk has enlarged its industry due to lactose intolerance, cow's milk allergy, and vegan lifestyles [53-62-77]. Hemp milk, with its highly nutritional value and low allergenicity, looks to be an attractive alternative to dairy, soy, and nut milks [53-62]. Comparison of milk products in the market reveals hemp milk as a better source of minerals than other dairy and plant milks [53-62-76]. Hemp milk does not taste that different but has a "nuttier" flavor in comparison to soy or rice milk [53-62]. High pressure homogenization following pH adjustment realized non- thermally processed hemp milk, which is remarkably stable, showing negligible phase separation in storage for 3 days at 4^{o}C [53-62-76]. Hemp is one of the ideal food materials because of its high protein content, low content of saturated fats, and high content of unsaturated fatty acids such as omega-3 and omega- 6 [1-53-62]. Addition of hemp protein concentrate significantly improved the nutritional value of the starch-based gluten-free bread [1-53-62]. Hemp also changed the rheological characteristics of the gluten-free dough and reinforced the structure [1-53-62]. Moreover, sensory acceptance on color and flavor was confirmed for the bread. Limited amylope recrystallization as well as limited hardening of the crumb were observed during storage [1-53-62]. Demand for high quality vegan meat made of plant materials is increasing based on the shortage of animal stock due to the global increase of human population, as well as in view of animal welfare [1-53-62]. Hemp cake, a residual material of oil expression from the hemp seed has long been utilized for livestock feeds [1-53-62-76]. It is a highly nutritive as well as sustainable feed stuff for cows, quails, cockerels, pigs, and broilers. Now, research is in progress to utilize hemp protein "directly" as a material for plant meat [1-53-62-76].

Zhou et al., (2024) [61] reported the effect of a single probiotic on the quality of hempseed fermented milk [61]. The addition of probiotics had little effect on titratable acidity, viable bacteria count, and textural properties but significantly increased the inhibition rate of α-glucosidase and pancreatic lipase [61]. GC-IMS conducted a general and validated analysis of the main flavor components, and a total of 19 important flavor compounds (with a relative content higher than 1%) were found to be the key factors in distinguishing the different aroma characteristics of hempseed milk fermented with four different probiotics [61]. Based on the results of LC-MS analysis, seven compounds were screened by correlation analysis for significant positive correlation with the hypoglycemic activities of the hempseed milk, including three flavonoids (panasenoside, genistein, and rutin), two amino acids (L-arginine and L-lysopine) and two short peptides (N2-gamma-glutamylglutamine and glutaminylmethionine) [61]. Seven of the compounds showed a significant positive correlation with hypolipidemic activities, including five amino acids (L-arginine, L-tyrosine, N-methylL-glutamic acid, N-acetyl-L-phenylalanine, and 4-guanidinobutanoic acid) and three short peptides (glycylleucine, hydroxyprolyl-lysine, and tryptophylhydroxyproline) [61]. This work by Zhou et al., (2024) [61] provides a detailed understanding of the effect of various probiotics on volatile and non-volatile metabolomic characteristics of hempseed milk and may provide a reference for the development of functional plant-based fermented milk [61].

Plant-based milk is becoming an increasingly important component in developing new food items. Hemp seed has anti-inflammatory effects and a high protein content, which gives health advantages [53-76]. Hemp milk is a suitable alternative for those with allergies to milk protein and lactose or concerns about cholesterol [53-76] However, optimal extraction conditions for hemp milk are not well documented. This study by Thakur et al., (2024) [79] utilized a response surface methodology (RSM) based on a Central composite rotatable design (CCRD) to optimize the variables of soaking time (A), heating time (B), and temperature (C) for hemp milk production. The study by Thakur et al., (2024) measured levels of anti-nutrients such as phytic acid, tannins, and saponins, as well as nutrients like fat and protein[76]. Regression models were developed to analyze these factors, and their effectiveness was evaluated using a lack of fit test and the coefficient of variation (R2) [79]. The study found that soaking duration, extraction time, and temperature had a substantial ($p < 0\ 05$) influence on responses. The optimal soaking period, extraction time, and temperature for best hemp seed milk production are as follows: soaking period of approximately 9 h, heating time in a water bath for almost 60 min, and heating temperature of 48° C [79]. The experimental findings confirmed the accuracy of the predicted models within a satisfactory response range. Additionally, the results of this study by Thakur et al., (2024) [79] suggested that the formulated model is effective for enhancing milk production and achieving consistent quality[79]. The trend will be expected to impact the hemp milk market significantly by attracting new consumer bases ready to try plant-based protein powders that are rich in nutrition, thereby growing hemp milk's share in the functional foods and supplements category[79].

4. Hemp based Probiotic Energy Milk Drinks

Plant-based drinks as substitute for milk consumption are raising striking interest in the food industry. Soy and rice drinks are the most studied and successful milk substitutes. An untapped source is hemp seed, which is a powerhouse of nutrients bearing bioactive compounds, but the production of derived drink is limited[53-76]. Another impetus for exploring alternative options stems from the ongoing rise in population demands for additional food sources to combat hunger, ensure food security, enhance nutrition, and foster sustainable agricultural practices [53-76]. In recent times, consumers have redirected their focus from traditional dairy products to non-dairy alternatives derived from plants, such as rice milk, cashew milk, soy milk, almond milk, coconut milk, hemp seed milk, and sesame seed milk[53-76]. Hemp seeds, derived from the *Cannabis sativa L.* plant, are known for their high-quality edible oil, which is celebrated for its nutritional and therapeutic benefits[1-53-76]. These seeds are highly adaptable and used in various culinary contexts, whether they are eaten raw, cooked, or toasted [1-53-76]. More recently, both due to the increase of lactose intolerance all over the world and the health benefits of alternative sources, a growing interest for non-dairy milk leads consumers' demand toward novel functional beverages[53-76]. Development of foods having a low glycemic index, low in saturated fats, carbohydrates, and additives is important[53-76]. At the same time, bioactive compounds and high energy value derived from good fats and fibers are always demanded[53-76]. Considering this scenario, plant-based drinks as an alternative to cow's milk is a trending food market sector[53-76]. Soy and rice are extensively studied and worldwide distributed plant-based drinks. However, there is the need to exploit alternative and possibly healthier [53-76]. Hemp seed may represent a potentially functional vegetable source for milk substitutes [1-53-76]. Besides its nutritional advantages, it is characterized also by a low content of saturated fats and a good percentage of polyunsaturated fatty acids (PUFA) ω3 and ω6[1-53-76]. It is naturally free of cholesterol and has a low glycemic index[53-76]. Furthermore, hemp seed has striking in vitro and ex vivo antioxidant activity that is lacking in soybean and rice, which are the two most common sources for vegetable drinks [1-53-76]. Due to its high protein and magnesium content (but not calcium), hemp seed with soy and rice represents a good candidate as vegetal source comparable to macro elementary composition to cow's milk [53-76]. Indeed, mixing different types of drinks could be the solution to

obtain a drink formulation with the right nutritional and functional balance[53-76]. In addition, the aforementioned bioactive compounds and fermentation through probiotic strains could contribute to increase the value of these beverages by classifying them as functional [53-76]. On the other hand, the acidity (yogurt from cow's milk is fermented to pH 4.6, while vegetables drinks to less than 4 [5] and inhibitory factors (hydroxycinnamic acids, tannins) [6,7] could make vegetable drinks hard to be exploited in fermentation processes [53-76]. Hemp seed drinks market is small (185 million USD) due to the unpleasant taste and aspect, which limit its acceptance to the consumer[53-76]. The unpleasant taste is partially derived from the biogenic amines, such as cadaverine and putrescine, which are transferred to derivate products, and the non attractive aspect is because the drink is an unstable oil-in-water (O/W) emulsion system [53-76]. Moreover, hemp seed fermented products are missing from the market, because the vegetable source matrix is almost sugarless and microbial fermentation is hard to follow[53-76].

The growing interest for non-dairy milk is due not only to the increase in the market for food intolerance products, but also to that of functional products[53-76]. Most alternative beverages lack nutritional balance compared to cow 's milk but contain active functional components with properties that promote well-being [53-76]. Hemp seed drink is an example of plant-based products that has a low content of saturated fats, a good percentage of polyunsaturated fatty acids (PUFA) ω-3 and ω-6, naturally free of cholesterol, lactose-free, and low glycemic index[53-76]. Considering the protein fractions (65% edestin and 35% albumin), hemp seeds have a well-balanced amino acid composition and are particularly abundant in arginine[53-76]. Moreover, hemp seeds are rich in terpenes and terpenoid compounds, such as 1-(R)-α-pinene, Δ-3-carene, *β*-myrcene, and *β*-caryophillene [53-76]. These molecules have antioxidant and pathogen-antimicrobial effects, even if high concentration of these compounds is reduced by heath treatment during hemp seed drink production [53-76]. The global hemp seed drink market was approximately USD 185 million in 2018 [53-76]. Nevertheless, hemp seed drink is still not widespread, according to the report of Zion Market Research. Reasons could lie on the generally unpleasant taste and appearance that limit consumer's acceptance[53-76]. Indeed, the hemp seeds are rich in biogenic amines, such as cadaverine and putrescine that are transferable to derivate products [53-76]. The drink is an unstable oil-in-water (O/W) emulsion system, easy to flocculate, coalesce, and cream that determine a loss of quality [53-76]. As done by several companies producing plant-based drinks, the mixtures of different type drinks could be the solution to meet the right nutritional balance, taste and texture[53-76]. For example, according to the nutritional values a mix of soy drink, rice drink and hemp drink could be comparable to cow's milk due to similar proteins, sugars and lipid content, respectively [53-76]. In addition to the nutritional balance, the bioactive compounds and fermentation by probiotic strains would contribute to increase the value of these foods by classifying them as functional [53-76]. Fermentation of plant-based drinks is mainly conducted with lactic acid bacteria, in particular *Streptococcus* spp. and *Lactobacillus* spp [53-76]. These bacteria are able to resist and grow in difficult environment, such as that of plant-based . In these drinks the extreme acidity (yogurt from cow's milk is fermented to pH 4.6, while plant-based drinks to less than pH 4) and the presence of inhibitory factors (hydroxycinnamic acids, tannins; are making the matrix hard for microbial fermentation[53-76]. mong *Lactobacillus* spp., *Lactobacillus plantarum* PH04 is a beneficial microbe, that could be commercially claimed as probiotic *Lb. plantarum* and *Lb. fermentum* harbor different alpha-glucosidases that make them ideal to ferment plant-based drinks such as soy[53-76].

In one of the study reported about introducing new formulations of commercial hemp seed-derived drink to be fermented with probiotics (*Lactobacillus fermentum*, *Lb. plantarum* and *Bifidobacterium bifidum*). In this work for the first time the prebiotic activity of different hemp seed drinks was assessed by cultural and quantitative PCR methods[53-76]. In addition, to better describe the prebiotic potential, VOCs alkenes and volatile organic acids were characterized by a metabolomic approach via GC-MS SPME[53-76]. Obtained results showed that the hemp seed drinks had strong prebiotic activity, ability to support probiotics growth and to increase the content of some bioactive compounds[53-76]. These outputs are in part due to the presence of different terpenes that inhibit the growth of enteropathogens and to high levels of acetate, propionate and butyrate produced during fermentation that support the growth of probiotics [53-76]. Although the health potential of hemp seed is well known, derived drinks are hitherto scarcely transformed and distributed, thus this work could provide some basics to produce prebiotic and probiotic fermented hemp seed drinks[53-76].

Liu et al., (2024) [56] reported that hemp seeds are highly nutritious, containing about 25% protein [56]. Compared to other plant proteins, hemp seed protein offers distinct benefits: it closely resembles human protein, making it particularly suitable for human dietary needs ; its amino acid profile is well-balanced and meets the ideal standards set by the Food and Agriculture Organization, thus being recognized as a "high-quality complete protein" by nutritionists; it does not contain tryptophan inhibitors, which improves digestibility and absorption; it has a mild, fresh taste with no bean-like odor [10]; and it positively affects blood pressure, blood sugar levels, digestion, weight management, and immune regulation, while also offering anti-tumor properties and alleviating symptoms of anemia, hypoxia, and fatigue[56]. Despite its health benefits, hemp seed protein is often underutilized because hemp seed meal, a by-product of oil extraction, is commonly used as animal feed rather than being harnessed as a valuable protein resource[56].

Liu et al., (2024) [56] reported Plant-based foods generally fall into three categories: plant-based meat, plant-based milk, and plant-based eggs [56]. Among these, the plant-based yogurt market shows significant promise, with projections indicating it could reach USD 144 billion globally by 2029 [56]. Currently, the primary ingredients for plant-based yogurt include bulk agricultural products like soy, oat, pea, and rice proteins [56]. Additionally, medicinal and edible proteins, such as those found in hemp seed, plum seed, and jujube seed, which each contain abundant proteins, offer valuable alternatives [56]. These proteins not only serve as potential raw materials for plant-based yogurt but also possess specific physiological benefits[56].

Nissen et al., (2019) [55] are of the opinion that vegetal drinks as a substitute for milk consumption are raising striking interest in the food industry[55]. Soy and rice drinks are the most successful milk substitutes but are low in protein and fiber contents, are rich in sugars, and their cultivation systems are unsustainable; thus, alternative vegetal sources to resolve these limits must be found [55]. Nissen et al., (2019) [55] reported that a winning candidate could be hemp seed, which is a powerhouse of nutrients, is sugarless, rich in fiber and proteins, and little land and nutrients demanding[55]. The aim is to develop novel drinks obtained from hemp seeds mixed or not with soy and rice and fermented with probiotics (*Lactobacillus fermentum*, *Lb. plantarum*, and *Bifidobacterium bifidum*). The drinks were characterized for their microbial growth, by means of culture-dependent and -independent techniques, and for their volatilome, by means of solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) analysis[55]. The results of the study conducted by Nissen et al., (2019) [55] showed that hemp seed drinks have a specific aroma and its compounds are dependent on the type of formulation and to the probiotic used55]. For example, in hemp seed drinks, 2-heptanol, 2-methyl, 2,4-decadienal, 2-butanone, 3-hydroxy, 2,3-butanedione, and propanoic acid were fine descriptors of probiotics fermentations. Nissen et al., (2019) [55] reported that multivariate analysis of volatile metabolites and their correlation to some physiological parameters and nutritional values offered a novel approach to assess the quality of functional hemp drinks which could result in a decisional tool for industrial applications [55].

The development of foods that has a low glycemic index, low in saturated fats, carbohydrates and additives, and lactose-free, but have high energy value derived from good fats and fibers is fundamental for the well-being of healthy people and the intolerant ones [53-55-76]. Considering this scenario, the food sector of dairy products has found alternatives to cow's milk with vegetable drinks[53-55-76]. Otherwise, the most common vegetable sources exploited, soy and rice, are steering a massive market and the feed stocks look not sufficient nor sustainable to satisfy the global demand, as demonstrated by the recent intense Amazon forest burnings or by the rising custom duties [53-55-76]. The search for alternative vegetable matrices is open and wide and a winning candidate could be hemp seed, which has a high nutritional value and generates higher yields in smaller fields [53-55-76]. Although health and nutritional potential of hemp seed are well known, the vegetable drink derived is still not common, because is less appetible for the consumer due to less pleasant taste, aspect, and aroma [53-55-76]. The study conducted by Nissen et al., (2019) [55] has improved the hemp seed drink, mixing with soy or rice drinks and was conducted fermenting with probiotic. For the first time, a metabolomic approach to study the sensorial characteristics of different hemp drink formulations is proposed. The results of the study conducted by Nissen et al., (2019) [55] showed that specific aromatic compounds are dependent not solely to the type of formulation used, but even to the probiotic inoculated [53-55-76]. The hemp seed drinks that presented have a specific aroma characterized by high abundances of heptanol, heptenal (Z), 2,3-butandedione, octadecane, caprylic acid, and pelargonic acids. This study by Nissen et al., (2019) [55] for the first time offers the metabolomic profiles of the aromatic VOCs of different hemp seed fermented drinks, in order to indicate possible solutions to obtain the best or desired formulations [53-55-76].

Hemp milk is a popular and convenient plant-based beverage with a nutrient profile similar to cow's milk but without cholesterol, gluten, or lactose[80]. Despite its benefits, hemp milk faces stability challenges related to its oil-in-water emulsion fermentation and germination, have emerged as promising alternatives to synthetic stabilizers, offering cost-effectiveness, environmental friendliness, safety, sustainability, and ease of implementation [80]. Hemp seeds contain prebiotic ingredients such as oligosaccharides, fibers, polyphenols, and isoflavones. Solid-state fermentation aids in the partial breakdown of organic substances, such as complex protein and protein-phenol structures, yielding smaller, soluble forms [80].

In another development, one of the study conducted by Karabulut et al., (2024) [80] introduces the concept of developing a functional hemp drink enriched with γ-Aminobutyric acid (GABA) to enhance its nutritional value and functional properties utilizing Solid-State (SSF) co-Fermentation by *Lactobacillus casei* and *Bacillus subtilis* and germination bioprocesses [80]. Bioprocesses may offer an alternative solution to challenges in hemp milk, such as product instability and the use of additives. Notably, the hemp milk produced through the germination for three days or co-fermentation processes yielded the highest GABA content of 79.84 and 102.45 mg/100 mL, respectively, compared to the untreated milk. [80]. These bioactive milk samples exhibited higher zeta potential and soluble protein content and also reduced solid particle sedimentation and droplet sizes ($D_{4,3}$ and $D_{3,2}$) compared to the untreated milk.

Furthermore, the peptide, total phenolic content, and antioxidant activity of the produced GABA-enriched kinds of milk surpassed those of the untreated milk [80]. Overall, the SSF and germination processes present a promising alternative for producing stable milk analogs with enhanced health-boosting properties [80].

The main problems associated with the fermentation of plant beverages are related to the sensory quality of the final product and to the resistance of probiotic microorganisms. Producers encounter difficulties with the physical stability caused by milk coagulation (it occurs at the beginning or in the course of storage). The appearance of these products resembles that of low-fat yoghurt [53-79]. Additional problems concern the survivability of probiotic bacteria, which is dependent on multiple factors, including e.g., presence of other microorganisms in the product, time and conditions of strains culture and product storage, product processing technology or pH value.

This study conducted by Szparaga et al., (2019) [82] aimed at determining the survivability of probiotic bacteria cultures in model non-dairy beverages subjected or not to the fermentation and storage processes, representing milk substitutes [82]. The experimental material included milks produced from desiccated coconut and non-dehulled seeds of hemp (*Cannabis sativa* L.) [82]. The plant milks were subjected to chemical and microbiological evaluation immediately after preparation as well as on day 7, 14, and 21 of their cold storage [82]. This study conducted by Szparaga et al., (2019) [82] results proved that the produced and modified plant non-dairy beverages could be the matrix for probiotic bacteria [82]. The fermentation process contributed to increased survivability of *Lactobacillus* casei subsp. rhamnosus in both coconut and hemp milk. During 21-day storage of inoculated milk substitutes, the best survivability of *Lactobacillus casei* was determined in the fermented coconut milk [82]. On day 21 of cold storage, the number of viable *Lactobacillus casei* cells in the fermented coconut and hemp milks ensured meeting the therapeutic criterion [82]. Due to their nutritional composition and cell count of bacteria having a beneficial effect on the human body, the analyzed groceries—offering an alternative to milk—represent a category of novel food products and their manufacture will contribute to the sustainable development of food production and to food security assurance [82].

5. Conclusion

Probiotics are defined as vital microorganisms which when ingested in suitable quantity offer a health benefit to the host**.** Probiotics are live microorganisms **(***Lactobacillus, Bifidobacterium***,** and a yeast, *Saccharomyces boulardii*) that can have beneficial effects on or inside body. Indian probiotic foods have a fascinating history deeply rooted in the country's rich cultural heritage. Many probiotic products are formulated with beneficial bacteria (*Lactobacillus, Bifidobacterium*) and yeasts (*Saccharomyces boulardii*) for the purpose of preventing or recovering from bacterial or yeast infections in our different body parts, including, atopic dermatitis and acne, cavities and gum diseases, vaginal and urinary tract infections, and antibiotic associated diarrhoea. It may also help to ease the symptoms of irritable bowel syndrome (IBS) and some other conditions. The growing interest for non-dairy milk is due not only to the increase in the market for food intolerance products, but also to that of functional products. Most alternative beverages lack nutritional balance compared to cow's milk but contain active functional components with properties that promote well-being. Hemp seed drink is an example of plant-based products that has a low content of saturated fats, a good percentage of polyunsaturated fatty acids (PUFA) ω-3 and ω-6, naturally free of cholesterol, lactose-free, and low glycemic index. Plant-based milk is becoming an increasingly important component in developing new food items. Hemp seed has anti-inflammatory effects and a high protein content, which gives health advantages. Hemp milk is a suitable alternative for those with allergies to milk protein and lactose or concerns about cholesterol.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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